ARL-TR-7185 ● JAN 2015

US Army Research Laboratory

Biodiversity of Aerosolized Particles (Final Report)

by Christian J Sund, Steven C Hill, David C Doughty, Matthew D Servinsky, Elliot Gerlach, Brandy Dorsey, Suelynn Ren, Edward D Creegan, Mark Coleman, Brian Reen, David C Ligon, Deryck D James, and Chatt C Williamson

NOTICES

Disclaimers

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Citation of manufacturer’s or trade names does not constitute an official endorsement or approval of the use thereof.

Destroy this report when it is no longer needed. Do not return it to the originator.

ARL-TR-7185 ● JAN 2015

US Army Research Laboratory

Biodiversity of Aerosolized Particles (Final Report)

by Christian J Sund, Steven C Hill, David C Doughty, Matthew D Servinsky, Elliot Gerlach, Brandy Dorsey, Suelynn Ren, Edward D Creegan, Mark Coleman, Brian Reen, David C Ligon, Deryck D James, and Chatt C Williamson

Sensors and Electronic Devices Directorate, ARL

Computational and Information Sciences Directorate, ARL

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED (From - To)
January 2015	DRI	10/2012–10/2014

4. TITLE AND SUBTITLE
Biodiversity of Aerosolized Particles (Final Report)

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Christian J Sund, Steven C Hill, David C Doughty, Matthew D Servinsky, Elliot Gerlach, Brandy Dorsey, Suelynn Ren, Edward D Creegan, Mark Coleman, Brian Reen, David C Ligon, Deryck D James, and Chatt C Williamson

5d. PROJECT NUMBER
FY13-CIS-002

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
US Army Research Laboratory
ATTN: RDRL-CIE-S
2800 Powder Mill Road
Adelphi, MD 20783-1138

8. PERFORMING ORGANIZATION REPORT NUMBER
ARL-TR-7185

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

An overarching objective of this Director's Research Initiative (DRI) is to explore the use of state-of-the-art DNA-sequencing techniques to begin to answer important questions about aerosolized microbes and other biological particles in the atmosphere, such as how these bioparticles vary with time, location, and air-mass trajectories and their associated humidity, rainfall, and land surface types. Our initial plan, to collect the air samples using Davis Rotating Unit for Monitoring (DRUM) samplers, failed to obtain sufficient DNA for sequencing. Methods were developed to isolate DNA from high-volume (hi-vol) samplers. We then obtained approximately 150 air samples with hi-vol samplers and 850 samples with PS-1 air samplers of the type commonly used by the US Army Public Health Command. Both samplers can obtain sufficient DNA for sequencing in times as short as 2 h. We computed air-mass trajectories, rainfall humidity, and temperature along each trajectory using Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT). These trajectories were used in picking the samples to sequence. DNA amplification and purification protocols were developed to target specific regions of prokaryotic and eukaryotic genomes for sequencing.

15. SUBJECT TERMS
bioaerosols, sequencing, genomics, Director's Research Initiative (DRI)

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	UU	30	Steven C Hill
Unclassified	Unclassified	Unclassified			19b. TELEPHONE NUMBER (Include area code) 301-394-1813

Contents

List of Figures

1. Introduction

DNA sequencing techniques have improved tremendously in the past few years. An overarching objective of this Director's Research Initiative (DRI) is to explore the use of rapidly improving state-of-the-art DNA-analysis techniques (commonly referred to as "next generation sequencing") to begin to answer important questions about aerosolized microbes and other biological particles in the atmosphere. Biological particles in the atmosphere, such as bacteria, viruses, fungal spores, and pollens (Lighthart et al. 1997, Bowers et al. 2011, Frohlich-Nowoisky et al. 2012, Hameed et al. 2009), can impact Soldier health by causing infections, asthma, allergies, or other pulmonary problems (Yamamoto et al. 2012). Additionally, they can impact weather by acting as condensation nuclei and by absorbing and emitting radiation. Some questions about the relative abundances of airborne bioparticles (including pathogenic and allergenic organisms) are the following:

- How do these vary with space, time, air mass trajectory, humidity, and temperature?
- How do they relate to particular biomes or to local conditions (soils, vegetation, and human activity)?

A long-term objective is to contribute to understanding bioaerosol threats to Soldier health and develop monitoring and mitigation strategies for bioaerosols. Such strategies are needed to begin to answer questions such as the following:

1) Are Soldiers deployed to southwest (SW) Asia exposed to significant numbers of airborne microorganisms and fungal spores that are different from those in the US?
2) How do the abundances of the airborne microorganisms and fungal spores vary with location?
3) Can these differences between locations be understood, at least partially, by the climate, biome, and winds?

Another objective is to obtain information on the bioaerosol backgrounds in different locations, which could aid developers of biowarfare agent detection systems. A better understanding of how bioparticles vary—by location, time, winds, etc.—is needed for identifying strategies to minimize negative impacts to the Soldier.

The approach is to do the following:

1) Collect aerosol samples containing the bioparticles (bioaerosols).

2) Obtain best estimates of the winds, air mass trajectories, and meteorological parameters associated with each sample using our local measurements, local and national databases, and the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) and Weather Research and Forecasting (WRF) modeling programs.

3) Isolate the DNA from each sample.

4) Selectively amplify the polymerase chain reaction (PCR) in the DNA near evolutionarily conserved regions of genomes (the DNA coding for 16S ribosomal RNA (rRNA) of bacteria, the 18S rRNA of eukaryotes and the internal transcribed spacer (ITS) regions of fungi).

5) Determine the sequences of these amplicons (approximately 6×10^6 100-base-pair reads are obtained for each of 96 samples by collaborators at the Edgewood Chemical Biological Center [ECBC] using their Illumina Hi-Seq).

6) Assign each sequence to its most likely phylogenetic categories (phylum to genus, depending upon the organism) using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline for taxonomy assignments (Caporaso et al. 2012), cluster the sequences, select some of these clusters to be operational taxonomic units (OTUs), generate trees and networks from the data, and quantify the relative abundance of sequences in different OTUs.

7) Use the weather data and sequence data for all the samples to ask questions regarding how the overall biodiversity and the occurrence of different sequences in the bioaerosols varies and what, if any, statistically significant variations are observed with air mass trajectories, source location regions (e.g., Florida vs. the Atlantic Ocean vs. Nebraska), and meteorological parameters. An advantage of this approach is that many more samples can be analyzed than with less capable techniques.

The data produced through this effort could provide information regarding differences in airborne microorganisms in the US vs. SW Asia and the Horn of Africa to the US Army Public Health Command (USPHC) and others studying health effects of deployed Soldiers. Understanding such correlations may help lead to methods of predicting the concentrations of various bioaerosols depending

upon air mass trajectories and the meteorological conditions along these trajectories.

The study has only partially been completed. The initial delays occurred because the DNA content of the samples collected with the Davis Rotating Unit for Monitoring (DRUM) samplers was too small to be analyzed effectively without tremendous amplification. This paucity of DNA was observed even with samples collected over 6-week periods. We were planning to perform this study using the DRUM samples in part because US Army Research Laboratory (ARL)/Battlefield Environment Division (BED) has significant experience with sample collections with these DRUMs in SW Asia and other locations. We switched to collecting samples using high-volume (hi-vol) and PS-1 samplers in order to obtain sufficient sample in shorter times. The later delays occurred because of difficulties in amplifying ribosomal DNA (rDNA) from these complex environmental samples for sequencing. These issues have been largely resolved as described in Section 4. Selectively amplifying the 16S rDNA in the presence of excess 18S rDNA was found out to be more difficult than we initially thought. That problem, combined with our use of the samplers, which are not size selective, probably reduces our ability to detect bacteria that are in relatively low concentrations. We have methods to further enhance the 16S to 18S ratio if that is desired. Also, the particulate samplers routinely used by USPHC (the PS-1) are not size selective, and so it is useful to develop techniques that work with such samples.

Our plan is to continue this work, obtain the DNA sequences, assign these sequences to taxa, and examine the data for relations between the taxa obtained and the wind trajectories, land or water types, and atmospheric conditions.

2. Air Sampling Techniques, Samples Collected and Evaluation of Adequacy

We first collected aerosols as stated in the DRI proposal, i.e., using the DRUM sampler. These samplers collect size- and time-dependent samples. They have been used by BED and contractors to collect particles in theater. Samples were collected using DRUM samplers for 6 weeks near Bldg. 202 (Adelphi Laboratory Center [ALC]) twice during the winter of 2012–2013. Several DNA isolation procedures were used to obtain DNA from these samplers. None of the techniques yielded sufficient DNA for sequencing reactions, even when all the particles collected in 6 weeks in 2 of the 8 size bins were combined.

To ensure that sufficient DNA is collected, and is collected quickly enough that variations in bioparticle concentrations in times as short as a few hours can be

examined, we collected particles using hi-vol samplers. These samplers do not differentiate by size. However, we found that they sample fast enough to obtain sufficient DNA in times as short as 2 h. We collected 124 samples using hi-vol samplers between April and February 2014 (Fig. 1). The primary hi-vol sampler was collocated with an automated meteorological sensor measuring wind speed and direction, temperature, and humidity for the majority of the samples taken.

Fig. 1 Photographs of 2 of the locations at which samples were taken. Each shows the hi-vol sampler used. The upper photo also shows the wind, temperature, and humidity sensor used.

In February 2014, we sent filters to Iraq to be used by Soldiers working with the USAPHC to collect air samples using PS-1 samplers. The filters reached the base, but unfortunately no samples were taken.

Because PS-1 samplers are used by USAPHC, we did our last sets of collections (approximately 100 samples) at ALC using these samplers.

A histogram of the sample length (in hours) for the first series of samples in 2013 and February 2014 is illustrated in Fig. 2.

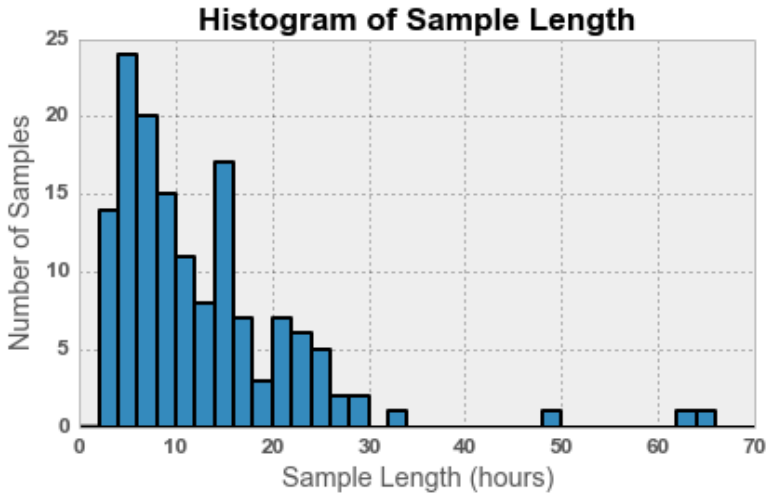

Fig. 2 Histogram of the sample lengths (h) used for the first series of samples

3. Air Trajectories, Meteorological, and Air Quality Conditions during Sampling Campaign

3.1 Air Mass Trajectories Using HYSPLIT

Air mass trajectories were computed using HYSPLIT (Draxler and Hess 1997, 1998, Draxler 1999). We select this method because it is frequently used for dust and smoke predictions (Stajner et al. 2012), is often used in bioaerosol studies (e.g., García-Lastra et al. 2012, Sadays et al. 2014, Morris et al. 2013, Urbano et al. 2011 Dannemiller et al. 2014), and generally performs similarly to more complex back-trajectory schemes, although more in-depth representation of boundary layer turbulence would be needed for source area computations (Hegarty et al. 2013, Koracin et al. 2011). Examples of air mass trajectories calculated using HYSPLIT for 2 of these samples (Fig. 3) illustrate trajectories that differ in source regions (southwest vs. north and east), time over the ocean (none or about 1 day), and rainfall (extensive or none). HYSPLIT trajectories were calculated for each of the 136 samples collected for the first set of

sequencing runs. These trajectories were used as one of the factors in prioritizing the samples for sequencing.

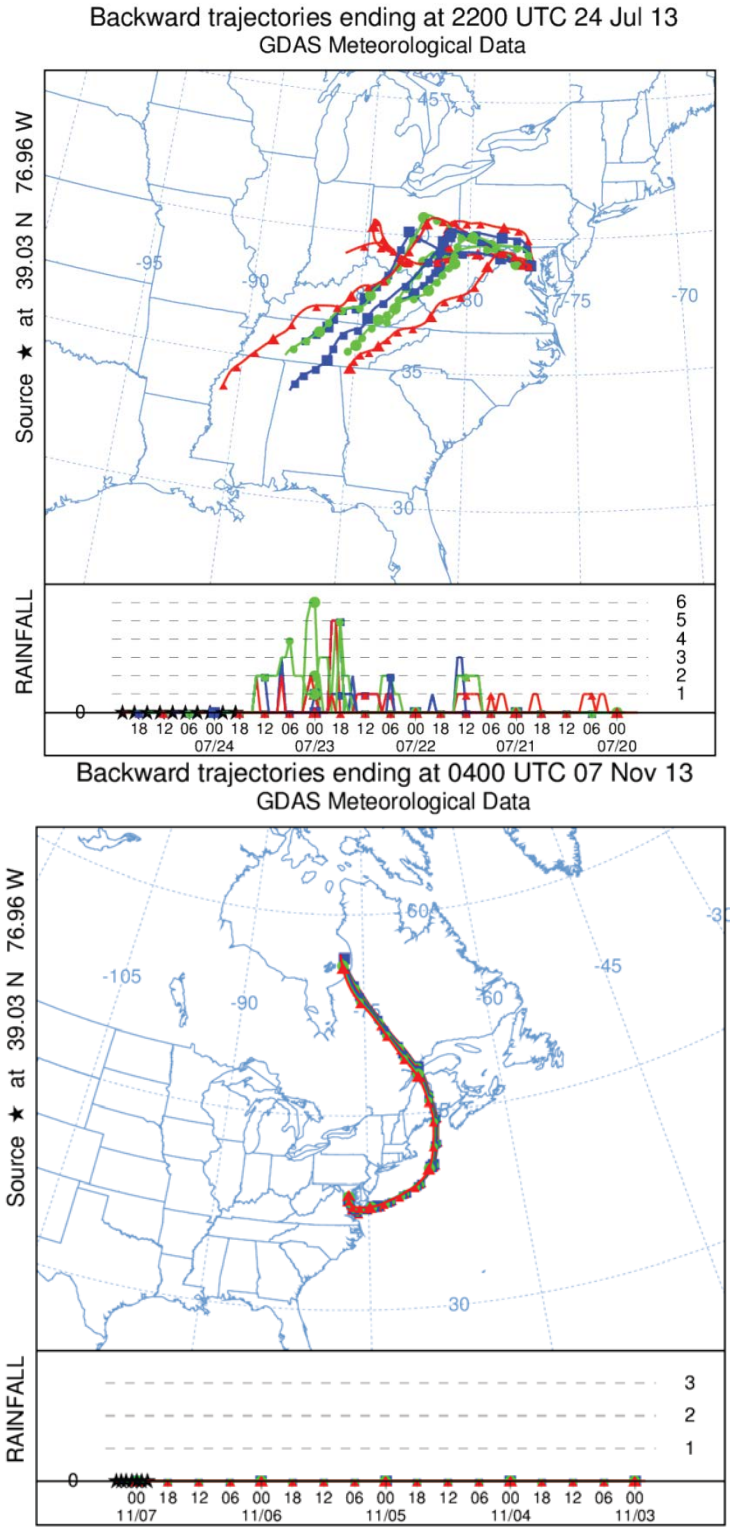

Fig. 3 Air mass trajectories calculated using HYSPLIT for 2 of the samples collected with the hi-vol sampler

3.2 Meteorological and Air Quality Conditions

3.2.1 Sampling Campaign Overview

Concentrations of different types of fluorescent bioaerosols are known to fluctuate during the day. For example, shortly before sunrise, an increase in bioaerosols may be due to nocturnal spore emission (Huffman et al. 2012, Rockett and Kramer 1974). Others have reported midday increases (Hameed et al. 2009). This change may be common to various terrestrial locations (Lighthart 1997). As a result, it is important to sample during different times of day.

Seasons also are known to affect the levels of bioaerosol (Jones and Cookson 1983), and different levels of biodiversity as determined by sequences have been observed in different seasons (Dannemiller et al. 2014). Meteorological conditions are also important, as both the amount of rainfall (Huffmann et al. 2013, Schumacher et al. 2013) and relative humidity (Shumacher et al. 2013, Jones and Harrison 2004), are correlated with changes in bioaerosols (concentrations of different types). Thus, it is critical to sample over a wide variety of meteorological conditions. The results presented in the following sections cover the first set of aerosol sampling from April 2013 to February 2014. Meteorological and air quality data are not available for all samples during the entire period, and so the number of actual data points for each meteorological or air quality parameter may vary.

3.2.2 Meteorological Conditions

Relative humidities (RHs) ranged from 20% to above 98% during the sampling campaign (Fig 4). There was no significant seasonal change in the observed levels of RH, other than that the lowest RH values were observed during the wintertime campaigns. Temperature ranged from above 30 °C to –5 °C during a very cold winter (Fig. 5). Locally, winds were primarily out of the west and north-northwest (Fig. 6). Winds from both Beltsville and College Park, Maryland, were analyzed due to uncertainties in the validity of wind data. Interestingly, although local winds were primarily out of the west, synoptically, air masses originated from many different locations and surface types (as described in Section 3.3)

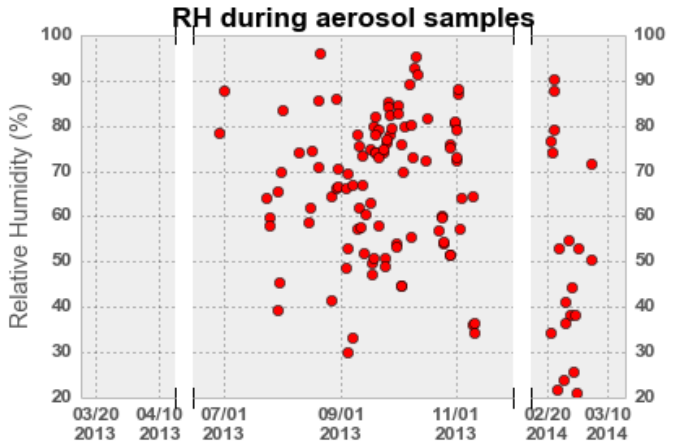

Fig. 4 RH from the Adelphi, Maryland, area during the sampling campaign

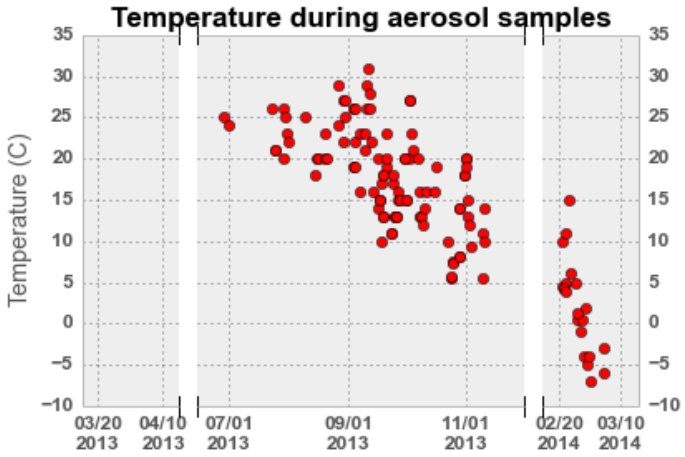

Fig. 5 Temperature (°C) from the Adelphi, Maryland, area during the sampling campaign

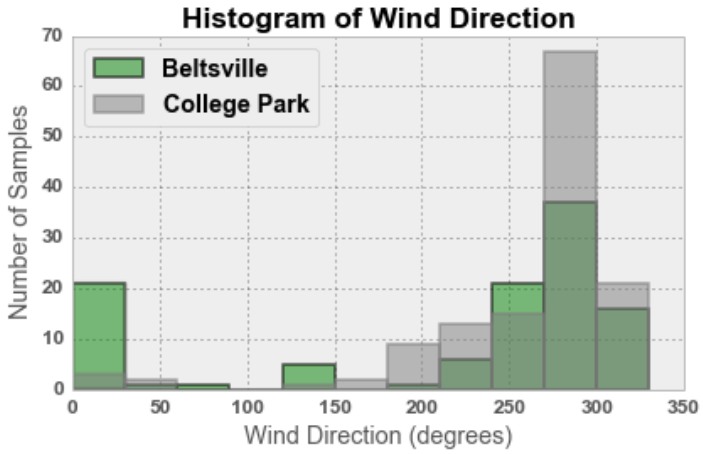

Fig. 6 Wind direction histogram from Beltsville, Maryland, (green) and College Park, Maryland, (translucent gray) during the sampling campaign

3.2.3 Air Quality Indicators

Because the majority of the hi-vol samples were collected from August through November 2013 and in February 2014, some atmospheric contaminants (ozone, PM_{10}) were relatively low during this time period. None of the periods exceeded the Environmental Protection Agency's (EPA) 24-h limit for either ozone or PM_{10} (particulate matter with a diameter of less than 10 microns). It is relevant that the highest levels of ozone are observed in July through September, as during this period, both the levels of oxidants available for reaction and the levels of sunlight that cause the ozone-producing photochemical reactions, are the highest. In general, PM_{10}, as seen in Fig. 7, was below 15 µg/m^3, so ambient aerosols, as determined by the filter samples, were low from a regulatory perspective.

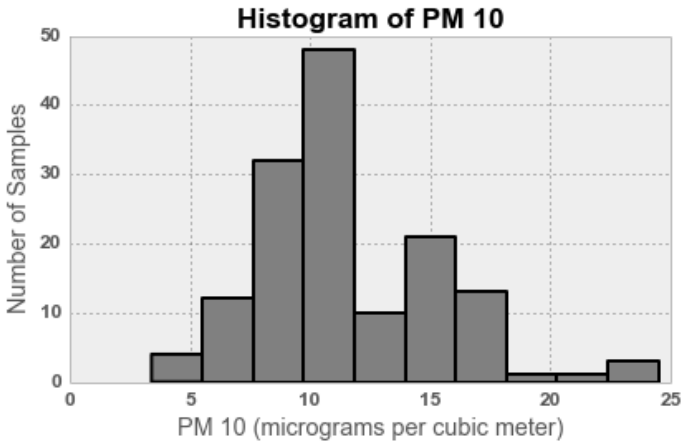

Fig. 7 Average sample PM_{10} levels during the sampling campaign. The EPA National Ambient Air Quality Standard for PM_{10} is 150 µg/m^3 for a 24-h average.

3.3 Determination of High-Resolution Back-Trajectories Using the Weather Research and Forecast Model

The influence of long-distance transport on the observed aerosols is uncertain. To better understand this uncertainty, and the potential factors which contribute to the atmospheric aerosol biodiversity, we applied several modeling techniques. We investigated the calculated back-trajectories using 3 different model outputs: from a nested model with a high-resolution inner nest, a regional model, and a global model. The WRF (Skamarock et al. 2008) was integrated with nested grids with 27-, 9-, 3-, and 1-km horizontal grid spacing centered over Adelphi, Maryland. The 9-, 3-, and 1-km grids are shown in Fig. 8. The National Center for Environmental Prediction's (NCEP) regional North American Mesoscale model (NAM) data we used have a horizontal grid spacing of 12 km. The Global Data Assimilation System (GDAS) data we used from NCEP have an approximate

resolution of 111x85 km at the latitude of Adelphi, Maryland, but is derived from a higher-resolution model.

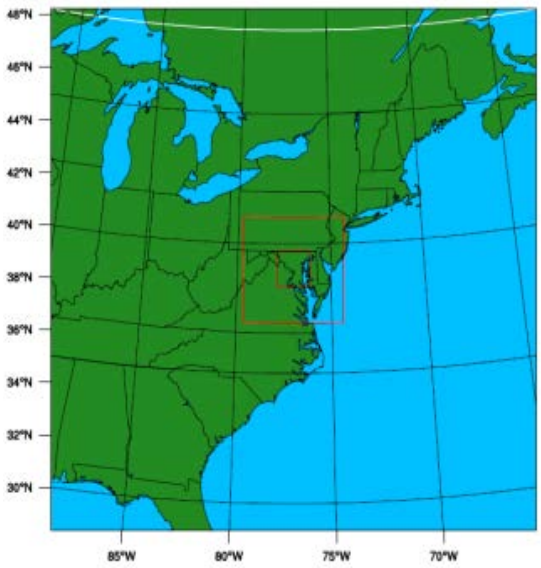

Fig. 8 WRF model domains for the 9-km (indicated by the extent of the figure), 3-km (indicated by the outer red box), and 1-km (indicated by the inner red box) horizontal grid spacing nests. The 27-km domain is much larger than the 3 smaller domains.

The WRF back-trajectories were computed using the Read/Interpolate/ Plot (RIP) program (http://www2.mmm.ucar.edu/wrf/users/docs/ripug.htm), while NAM and GDAS back-trajectories were computed using HYSPLIT. A comparison of WRF, NAM, and GDAS data for the 1400 and 2300 universal time coordinated (UTC) on September, 13, 2013, is shown in Fig. 9. The finer-resolution models appear to have more detailed features in the back-trajectories. Although the models generally agree for the initial (near Adelphi) trajectory, there is wide difference between them during the last few days (distant portion) of the back-trajectories. The end conclusion is that any further work should carefully evaluate the uncertainties, and should use ensembles of back-trajectories to ensure statistical significance of results.

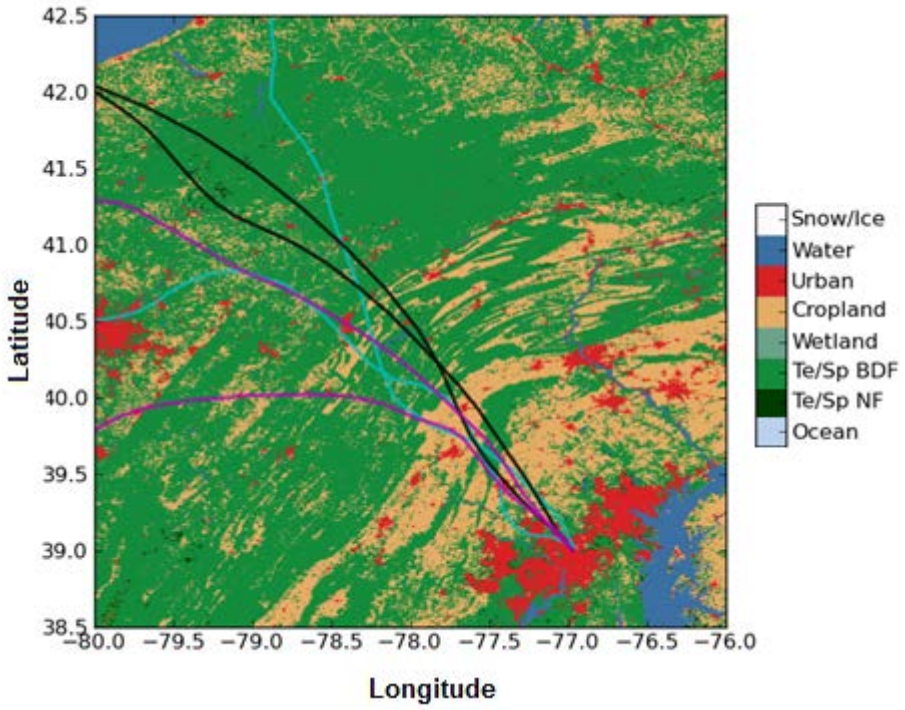

Fig. 9 Partial back-trajectories from the WRF (cyan), NAM (magenta), and GDAS (black) for 1400 and 2400 UTC on September 13, 2013, overlaid with land surface type

3.4 Source Region Analysis

During the sampling campaign, air masses at the site had originated from a wide variety of locations, with many different transport paths. These air masses passed over different kinds of land and ocean surfaces, which may have affected the content and character of the aerosols at the measurement location. To understand the influence(s) that air-mass trajectory has, we applied a time-weighted residence time analysis (TWRTA) using high-resolution (250-m) Geographic Information System (GIS) land surface data from the North American Land Change Monitoring System (NALCMS). Back-trajectories are overlaid with these data for illustrative purposes (Fig. 10). To expedite analysis, we computed the percentage of each number of trajectory sample points in 1° x 1° bins, and used that data to compute TWRTAs, in the manner of van Pinxteren et al. (2010).

We discuss 3 samples, each with its set of air-mass trajectories. These samples have, respectively, the majority of the influence from ocean (Fig. 10a), cropland (Fig. 10b), and forest (Fig. 10c). Each sample has approximately 4%–8% influence from urban areas. For each of the 7 trajectories in the "ocean" sample, oceans contribute between 36% and 55% of the TWRTA. The surface type, which contributes second most to the TWRTA, is either water or the temperate broadleaf

deciduous forest, depending on the individual trajectory. During the progression of the trajectory away from the sampling location, initially, forests and cropland were more important, but water increased after a slight decrease, as did the ocean influence. In summary, this sample is mostly ocean, with influences primarily from broadleaf deciduous forests, cropland, and coastal water.

The “cropland” case has between 50% and 60% cropland influence, with secondary influence (30.8%–42.7%) from broadleaf deciduous forests. Other minor influences (>0.1%) from urban, water, and grasslands are also observed. The “forest” case is essentially an inverse of the “cropland’ case, with 54%–55% influence from broadleaf deciduous forest, 39%–39% cropland, and 4% urban, with lesser influences from water, needleleaf forests, and wetlands.

Some general comments are in order. Primarily, back-trajectories during these samples spent the majority of the time over oceans, cropland, and broadleaf deciduous forests, with all other sources fairly minor. However, as urban areas are located in the immediate vicinity of the sample location, and the method applied used a linear weight (compared with an exponential weight), the influence of locations which are further away may be inflated.

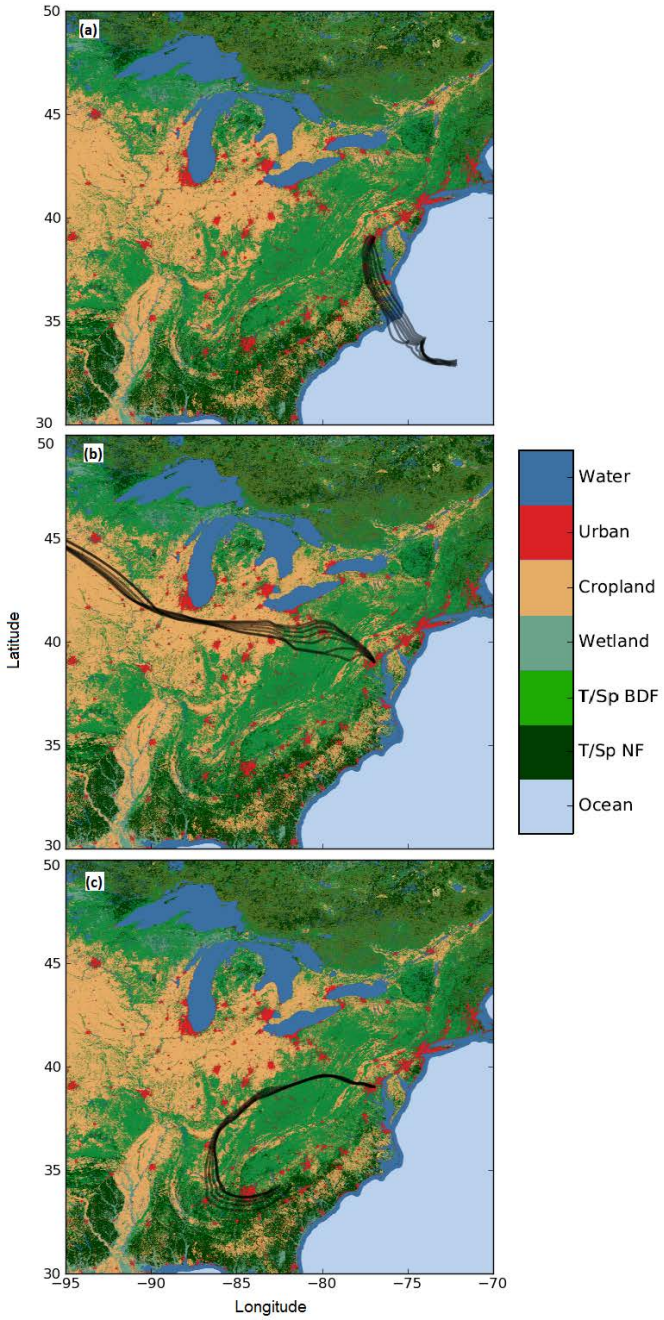

Fig. 10 Superposition of back-trajectories for three samples onto GIS data. High-resolution GIS NALCMS 250-m data. Major contributors include coastal and inland water (dark blue), urban (red), cropland (tan), wetland (olive), temperate/subpolar broadleaf deciduous forest (light green), and temperate/subpolar needleleaf forest (dark green).

4. DNA Extraction and Analysis Techniques and Results

We developed a protocol for isolation of DNA from hi-vol samples collected on quartz fiber filters. For DNA isolation, the filters were cut into small pieces and

these pieces were processed with a PowerSoil DNA isolation kit (MO-BIO). Sufficient DNA for downstream sequencing reactions was obtained using this approach. PCR amplification was used to determine if the isolated DNA was amenable to standard molecular biology techniques and establish if 16S DNAs and 18S DNAs were present in the samples. Figure 11 shows an agarose gel used to separate PCR amplified fragments of DNA isolated from filters. Both the 16S DNA from prokaryotic organisms and of the 18S DNA from eukaryotic organisms were observed using 18S primers, SSU_F04 and SSU_R22 , and 16S primers, 357F and 926R, as previously described (Sim et al. 2012, Bhadury et al. 2011, Bik et al. 2012). The primers used for these PCR reactions amplify the same region of the 16S and 18S genes that will be used for organism characterization.

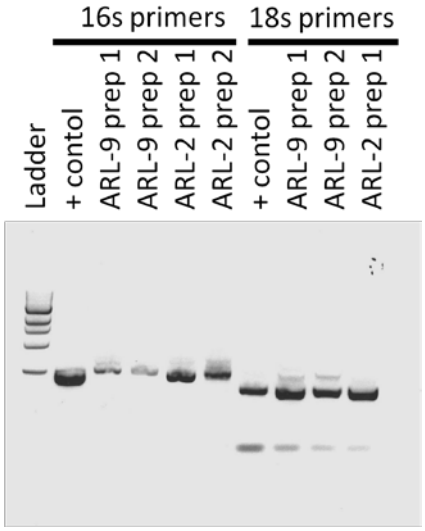

Fig. 11 Image of an agarose gel used to separate PCR reactions amplifying 16S DNA or 18S DNA. Two different preparations of DNA isolated from 2 different hi-vol filters were used as templates for amplification.

Ninety-six samples were PCR amplified using the primer sets described above and shipped to ECBC for next generation sequencing. Initial analysis by ECBC using an Agilent Bioanalyzer (Fig. 12a) indicated the samples contained a large amount of DNA that was not the appropriate target length. Sequencing of these samples would have resulted in high levels of background, reducing the number of reads of the target regions. To remove non-target DNA from the samples the PCRs were separated by gel electrophoresis (Fig. 12c) and the correctly sized bands were excised from the gels and purified. DNA fragments from several bands were sequenced using traditional Sanger sequencing to ensure the bands contained the target DNA sequences (data not shown). Purified samples were sent to ECBC for analysis using an Agilent Bioanalyzer. The results shown in Fig. 12b

indicated the purified samples were enriched for the target DNA (length) and were suitable for next generation sequencing. Ninety-six PCR amplified-, gel purified-18S samples were sent to ECBC for next generation sequencing.

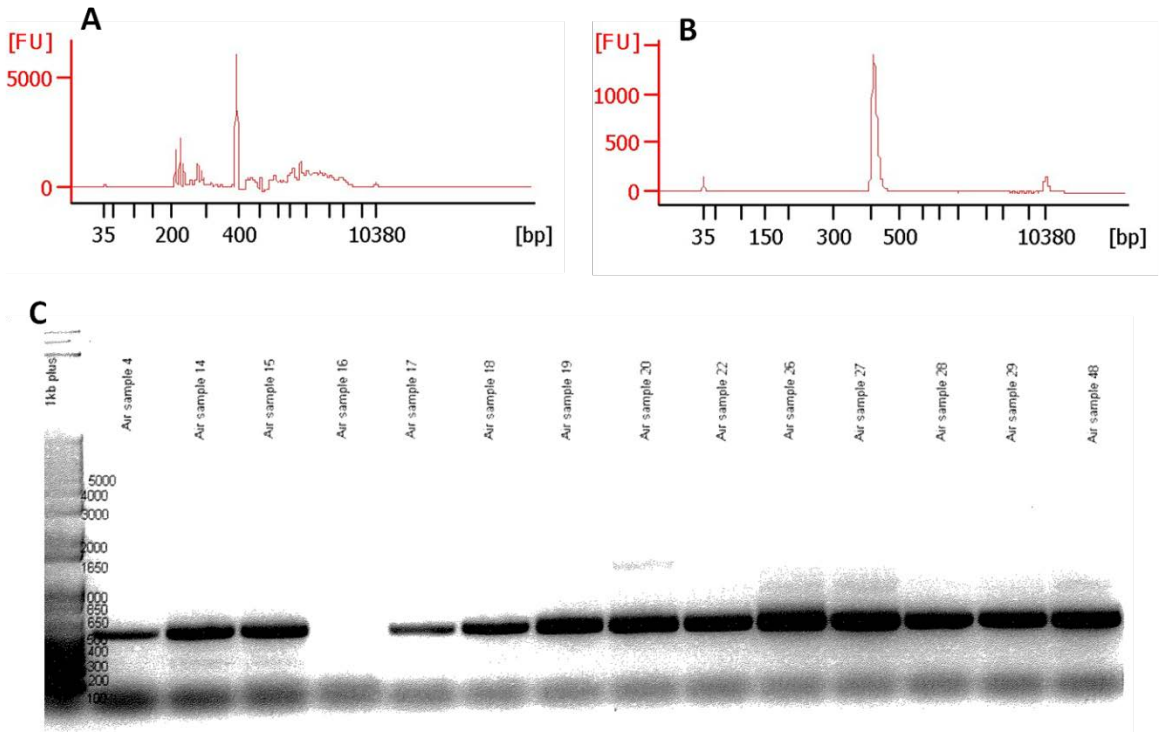

Fig. 12 Analysis of the PCR samples for sequencing analysis. a) Representative result from the analysis of the initial PCR samples using an Agilent Bioanalyzer. The x-axis depicts the length of the DNA and the y-axis is the fluorescent intensity, which correlates to amount of DNA. b) Representative result from the analysis of the gel-purified PCR products using an Agilent Bioanalyzer. c) Image of an agarose gel showing the PCR-amplified bands for 18S RNA genes. The DNA bands were excised and purified for further analysis.

5. Conclusion

We showed that to properly use back-trajectories to understand bioaerosol sources ensembles of trajectories with careful evaluation of uncertainties were required. We took bioaerosol filter samples when the measurement location was influenced by a wide variety of source locations, such as cropland, urban, ocean, and broadleaf deciduous forest. Such information, along with meteorological and air quality data, will be important in developing an understanding of the factors contributing to the bioaerosol background.

A DNA extraction technique was developed for isolating DNA from particles collected by hi-vol filters. Protocols for PCR amplification of target regions of prokaryotic and eukaryotic genomes were refined to ensure compatibility with

high throughput DNA sequence analysis. After protocol refinement, 96 samples were sent to ECBC for sequencing on an Illumina Hi-Seq sequencer and we are currently waiting for these results.

6. References

Bhadury P, Bik, Lambshead JD, Austen MC, Smerdon GR, Rogers AD. (2011). Molecular Diversity of Fungal Phylotypes Co-Amplified Alongside Nematodes from Coastal and Deep-Sea Marine Environments. PLoS ONE. 2011;6(10): e26445. doi:10.1371/journal.pone.0026445.

Bik H, Sung W, De Ley P, Baldwin J, Sharma J, Rocha-Olivares A, Thomas W. Metagenetic community analysis of microbial eukaryotes illuminates biogeographic patterns in deep-sea and shallow water sediments. Molecular Ecology. 2012;21(5):1048–1059, doi: 10.1111/j.1365-294X.2011.05297.x.

Bowers RM, McLetchie S, Knight R, Fierer N. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. The ISME Journal. 2011;5:601–612, doi: 10.1038/ismej.2010.167.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal. 2012;6:1621–1624, doi: 10.1038/ismej.2012.8

Draxler R. HYSPLIT 4 user's guide. Silver Spring (MD): NOAA Air Resources Laboratory; 1999 35 p. Report no.:ERL ARL-230.

Draxler R and Hess G. An overview of the HYSPLIT 4 modeling system of trajectories, dispersion, and deposition. Australian Meteorology Magazine, 1998; 47:295–308.

Draxler R, Hess G. Description of the HYSPLIT 4 modeling system. Silver Spring (MD): NOAA Air Resources Laboratory; 1997 24 p. Report No: ERL ARL- 224.

Dannemiller KC, Lang-Yona N, Yamamoto Y, Rudich Y, Peccia J. Y. Combining real-time fPCRg and next-generation fDNAg sequencing to provide quantitative comparisons of fungal aerosol populations. Atmospheric Environment. 2014;84:113–121, doi: 10.1016/j.atmosenv.2013.11.036.

Fröhlich-Nowoisky J, Burrows SM, Xie Z, Engling G, Solomon PA, Fraser MP, Mayol-Bracero OL, Artaxo P, Begerow D, Conrad R, Andreae MO, Després VR, Pöschl U. Biogeography in the air: fungal diversity over land and oceans. Biogeosciences. 2012;9:1125–1136, doi:10.5194/bg-9-1125-2012

García-Lastra R, Leginagoikoa I, Plazaola JM, Ocabo B, Aduriz G, Nunes T, Juste RA. Bluetongue Virus Serotype 1 Outbreak in the Basque Country (Northern Spain) 2007–2008. Data Support a Primary Vector Windborne Transport. PLoS ONE. 2012;7(3):e34421, doi:10.1371/journal.pone. 0034421.

Hameed AA, Khoder M, Yuosra S, Osman A, Ghanem S. Diurnal distribution of airborne bacteria and fungi in the atmosphere of Helwan area, Egypt. Science of the Total Environment. 2009;407:6217–6222, doi: 10.1016/j.scitotenv. 2009.08.028.

Hegarty, J, Draxler RR, Stein AF, Brioude J, Mountain M, Eluskiewicz J, Nehrkorn T, Ngan F, Andrews A. Evaluation of Lagrangian particle dispersion models with measurements from controlled tracer releases. Journal of Applied Meteorology & Climatology. 2013;52:2623–2637, doi: 10.1175/JAMC-D-13-0125.1.

Huffman JA, Prenni AJ, DeMott PJ, Pöhlker C, Mason RH, Robinson NH, Fröhlich-Nowoisky J, Tobo Y, Després VR., Garcia E, Gochis DJ, Harris E, Müller-Germann I., Ruzene C, Schmer B, Sinha B, Day DA, Andreae MO, J. Jimenez L, Gallagher M, Kreidenweis SM, Bertram AK, Pöschl U. High concentrations of biological aerosol particles and ice nuclei during and after rain. Atmospheric Chemistry and Physics. 2013;13:6151–6164, doi:10.5194/acp-13-6151-2013.

Huffman JA, Sinha B, Garland RM, Snee-Pollmann A, Gunthe SS, Artaxo P, Martin ST, Andreae MO, Pöschl U. Size distributions and temporal variations of biological aerosol particles in the amazon rainforest characterized by microscopy and real-time UV-APS fluorescence techniques during AMAZE-08. Atmospheric Chemistry and Physics. 2012;12:11997–12019, doi:10.5194/acp-12-11997-2012.

Jones AM and Harrison RM. The effects of meteorological factors on atmospheric bioaerosol concentrations - a review. Science of The Total Environment. 2004;326:151–180, doi10.1016/j.scitotenv.2003.11.021.

Jones B, Cookson J. T. Natural atmospheric microbial conditions in a typical suburban area. Applied and Environmental Microbiology. 1983;45:919–934.

Koracin D, Vellore R, Lowenthal DH, Watson JG, Koracin J, McCord T, DuBois DW, Chen L-WA, Kumar N, Knipping EM, Wheeler NJM, Craig K, Reed S. Regional source identification using Lagrangian stochastic particle dispersion and HYSPLIT backward-trajectory models. Journal of the Air & Waste Management Association. 2011;61:660–672, doi:10.3155/1047-3289.61.6.660.

Lighthart B. 1997: The ecology of bacteria in the alfresco atmosphere. FEMS Microbiology Ecology. 1997;23:263–274, doi:10.1111/j.1574-6941.1997.tb00408.x.

Morris CE, Leyronas C, Nicot PC. Movement of Bioaerosols in the Atmosphere and the Consequences for Climate and Microbial Evolution. In: Colbeck I, Lazaridis M, editors. Aerosol Science: Technology and Applications. Chichester, UK: John Wiley & Sons, Ltd; 2013. John Wiley & Sons, Ltd. 2013. p. 393–415.

Rockett TR, Kramer C. Periodicity and total spore production by lignicolous basidiomycetes. Mycologia. 1974;66(5):817–829.

Sim K, Cox MJ, Wopereis H, Martin R, Knol J, Li M-S, … Kroll JS. Improved Detection of Bifidobacteria with Optimised 16S rRNA-Gene Based Pyrosequencing. PLoS ONE. 2012;7(3):e32543, doi:10.1371/journal.pone.0032543

Sadyś M, Skjath C, Kennedy R. Back-trajectories show export of airborne fungal spores (*Ganoderma* sp.) from forests to agricultural and urban areas in England. Atmospheric Environment, 2014;84:88–99, doi: 10.1016/j.atmosenv.2013.11.015.

Schumacher CJ, Pöhlker C, Aalto P, Hiltunen V, Petäjä T, Kulmala M, Pöschl U, Huffman JA. Seasonal cycles of fluorescent biological aerosol particles in boreal and semi-arid forests of Finland and Colorado. Atmospheric Chemistry and Physics. 2013;13:11987–12001, doi:10.5194/acp-13-11987-2013.

Skamarock WC, Klemp JB, Dudhia J, Gill DO, Barker DM, Duda MG, Huang X-Y, Wang W, Powers JG. A Description of the Advanced Research WRF Version 3. Boulder (CO): National Center for Atmospheric Research (US); 2008 125p. Report No: NCAR/TN–475+STR.

Stajner I, Davidson P, Byun D, McQueen J, Draxler R, Dickerson P, Meagher J. US national air quality forecast capability: Expanding coverage to include particulate matter. In: Steyn DJ, Castelli ST, editors. Air Pollution Modeling and its Application XXI, NATO Science for Peace and Security Series C; 2012. P. 379–384.

Urbano R, Palenik B, Gaston CJ, Prather KA. Detection and phylogenetic analysis of coastal bioaerosols using culture dependent and independent techniques. Biogeosciences. 2011;8:301–309, doi:10.5194/bg-8-301-2011.

van Pinxteren, D, Brüggemann E, Gnauk T, Müller K, Thiel C, Herrmann H A GIS-based approach to back-trajectory analysis for the source apportionment of aerosol constituients and its first application. Journal of Atmospheric Chemistry. 2010;67:1–28 doi: 10.1007/s10874-011-9199-9.

Yamamoto N, Bibby K, Qian J, Hospodsky D, Rismani-Yazdi H, Nazaroff WW, Peccia J. Particle-size distributions and seasonal diversity of allergenic and pathogenic fungi in outdoor air. The ISME Journal. 2012;6(10):1–11, doi:10.1038/ismej.2012.30.

List of Symbols, Abbreviations, and Acronyms

ALC	Adelphi Laboratory Center
ARL	US Army Research Laboratory
BED	Battlefield Environment Division
DRI	Director's Research Initiative
DRUM	Davis Rotating Unit for Monitoring
ECBC	Edgewood Chemical Biological Center
EPA	Environmental Protection Agency
GDAS	Global Data Assimilation System
GIS	Geographic Information System
hi-vol	high-volume
HYSPLIT	Hybrid Single-Particle Lagrangian Integrated Trajectory
ITS	internal transcribed spacer
NALCMS	North American Land Change Monitoring System
NAM	North American Mesoscale
NCEP	National Center for Environmental Prediction
OTUs	operational taxonomic units
PCR	polymerase chain reaction
QIIME	Quantitative Insights Into Microbial Ecology
RH	relative humidity
RIP	Read/Interpolate/ Plot
rDNA	ribosomal DNA
rRNA	ribosomal RNA
SW	southwest
TWRTA	time-weighted residence time analysis
USPHC	US Army Public Health Command

UTC	universal time coordinated
WRF	Weather Research and Forecasting

1 (PDF) DEFENSE TECHNICAL INFORMATION CTR DTIC OCA

2 (PDF) DIRECTOR US ARMY RESEARCH LAB RDRL CIO LL IMAL HRA MAIL & RECORDS MGMT

1 (PDF) GOVT PRINTG OFC A MALHOTRA

3 (PDF) DIRECTOR US ARMY RESEARCH LAB RDRL CIE S C SUND S HILL D DOUGHTY

INTENTIONALLY LEFT BLANK.